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Duck hepatitis B virus inactivation and 8-methoxypsoralen photoadduct formation in human platelet concentrates.

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Photochemical inactivation (PCI) of virus and bacteria in platelet concentrates (PC) has been demonstrated using 8-methoxypsoralen (8-MOP) and long-wavelength UV light (UVA). To study inactivation of blood-borne virus, we have employed duck hepatitis B virus (DHBV), a model for human hepatitis B virus. A specific hepatocyte culture infectivity assay, with PCR detection, could measure 5-6 log10 virus kill. The DHBV inactivation in PC was dependent on UVA dose, was enhanced when plasma was reduced from 100% to 20% and was limited by 8-MOP solubility in the reduced-plasma medium. Optimum conditions for PCI were 100 micrograms/mL 8-MOP in 20% plasma and 80% synthetic platelet storage medium. A radiolabeling assay for 8-MOP photoadducts in hepatocytes seeded into PC confirmed that DHBV inactivation reflected DNA modification and indicated that adduct formation was insensitive to minor variations in conditions. Kinetic modeling indicated that optimum adduct formation was a compromise between 8-MOP dark binding and optical transmittance and that plasma proteins competed for 8-MOP binding. The PCI results in various media correlated with corresponding DNA modification densities and were compared to statistical models incorporating DHBV characteristics and predictions of 8-MOP crosslink formation between DNA strands. Behavior was consistent with one or a small number of lethal modifications per DNA strand, including monoadducts, but probably not crosslinks alone. A minor subpopulation of DHBV was found to be somewhat more difficult to inactivate, consistent with three-fold lower modification, due possibly to single-stranded DNA character or host repair of photoadducts.

PMID: 9648535 [PubMed - indexed for MEDLINE]

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[Inactivation of BVDV (experimental model for hepatitis C) using low pH and heat treatment in intravenous human immunoglobulins]

[Article in Spanish]

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PURPOSE: To measure the capability of heat (60 degrees C for 10 hr) and low pH to inactivate BVDV (a model of HCV) in human intravenous immunoglobulins. MATERIALS AND METHODS: The study was carried out on three batches of immunoglobulins produced by the Cohn method and contaminated with a known amount of BVDV. These mixtures, with and without 33% sorbitol, were submitted to heat treatment at 60 degrees C for 10 hours. The same immunoglobulin batches were manufactured at pH 4.25 and 4.5 and stored at 4 degrees C and 4 degrees C and 21 degrees C for 28 days. Samples of the two experiments were taken at the beginning and the end. The viral infectiousness was calculated by the standard microtiration method in 96-well plates, using the CPE, and the reduction factor was measured for each experiment. RESULTS: Complete viral inactivation was achieved with the heat treatment after 4 hours, and the 33% sorbitol decreased the formation of aggregates. Treatment by pH 4.5, at 21 degrees C for 28 days, decreased the viral load by approximately 2 log; no viral inactivation was achieved in samples stored at 4 degrees C. CONCLUSION: Heat is an effective method for inactivating HCV in final batches of human intravenous immunoglobulins when 33% sorbitol is added. The use of low pH at 21 degrees C as a method of viral inactivation must be evaluated case by case, since, according to the present results, it only achieved a 2 log inactivation.

PMID: 10618912 [PubMed - indexed for MEDLINE]

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Size and conformational stability of the hepatitis A virus used to prepare VAQTA, a highly purified inactivated vaccine.

Volkin DB, Burke CJ, Marfia KE, Oswald CB, Wolanski B, Middaugh CR.

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A variety of biophysical techniques have been employed to examine the size and conformational integrity of highly purified hepatitis A virus (HAV) in solution (purified HAV particles are subsequently formalin-inactivated and adsorbed to aluminum salts for use as the vaccine VAQTA). The size of HAV particles was assessed by a combination of electron microscopy, sedimentation velocity, and dynamic light scattering. The effect of ionic strength and temperature on the overall conformational stability of HAV was determined by a combination of intrinsic HAV protein fluorescence, fluorescent probes of both RNA and protein, and UV-visible spectroscopy. A major structural change in HAV occurs near 60 degrees C with the addition of 0.2 M magnesium chloride enhancing the thermal stability of HAV by approximately 10 degrees C. Salt concentrations above 0.2 M, however, decrease the solubility of HAV. The effect of pH on the physical properties of HAV particles was monitored by dynamic light scattering, analytical size exclusion HPLC, and interaction with fluorescent dyes. HAV particles undergo a substantially reversible association/aggregation at pH values below 6 with the concomitant exposure of previously buried hydrophobic surfaces below pH 4. These results are in good agreement with previous studies of HAV thermal stability under extreme conditions in which the irreversible inactivation of the viral particles was measured primarily by the loss of viral infectivity. The wide variety of biophysical measurements described in this work, however, directly monitor structural changes as they occur, thus providing a molecular basis with which to monitor HAV stability during purification and storage.

PMID: 9188048 [PubMed - indexed for MEDLINE]

Set	Items	Description
S1	5423	(VIRUS OR VIRAL)(5N)INACTIVAT?
S2	842801	MEASURE? ? OR MEASUREMENT
S3	30	S1(5N)S2
S4	29	RD (unique items)